Molecular Medicine 5: 773-782, 1999

Molecular Medicine

Review Article

How Cells Use Proteolysis to Control Their Growth

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Introduction

Protein destruction may seem like a strange way for a cell to control its growth. The entire process by which cells multiply is based on synthesis—the duplication of almost every molecule in the cell. If protein synthesis is one of the foundations of growth, then why are so many proteins destroyed as one cell becomes two?

The answer to this question is control. The process of cell duplication is, of course, more complicated than simply replicating the cellular contents and dividing. Survival of all eukaryotic cells depends on their ability to regulate how and when they grow. To persist, cells must multiply only in response to specific signals, they must have mechanisms to detect errors in the duplication process, and they must establish a defined sequence for the events of duplication, so that DNA replication, for example, always precedes cell division. In addition to synthesis, therefore, an important part of growth is maintaining tight control over all of the proteins needed to make a new cell. Protein destruction—proteolysis—plays a pivotal role in controlling the activity of these proteins.

Proteolysis is particularly suited to controlling the events of cell duplication because it is a swift, direct, and irreversible way to limit the activity of a protein. When a protein is destroyed by proteolysis, its levels in the cell can drop instantly and dramatically. Through this property, milestones in the duplication process are defined, such as exit from mitosis. Moreover, once a protein is destroyed, the only way it can reappear is by new synthesis; this gives direction to the events of cell duplication, and ensures that these events fire in the proper sequence.

As any newcomer to the field can appreciate, the regulation of eukaryotic cell growth is a complex phenomenon. How and when a cell grows is ultimately the product of interactions among vast groups of transcription factors, kinases, phosphatases, and other regulatory molecules. Similarly, the ways in which these molecules are affected by proteolysis are also complex. There are many different substrate proteins, a diverse and complicated proteolytic machinery, and a variety of mechanisms that modulate interactions between the two. Despite this complexity, however, the fundamental ways in which proteolysis works are surprisingly simple: nature has made the most of just a handful of strategies. In this review, I discuss how these simple strategies for using and controlling the power of proteolysis dominate many aspects of eukaryotic cell growth.

Proteolysis and Growth Control

To understand the simple ways in which cells put proteolysis to work to control their growth, the story needs to get a little complicated. We need to understand how cells structure the events required for their duplication, and how proteolysis works. We also need to understand how these two areas intersect and how the destruction of particular proteins relates to growth control. Once these more complicated issues have been discussed, the simple themes behind the function

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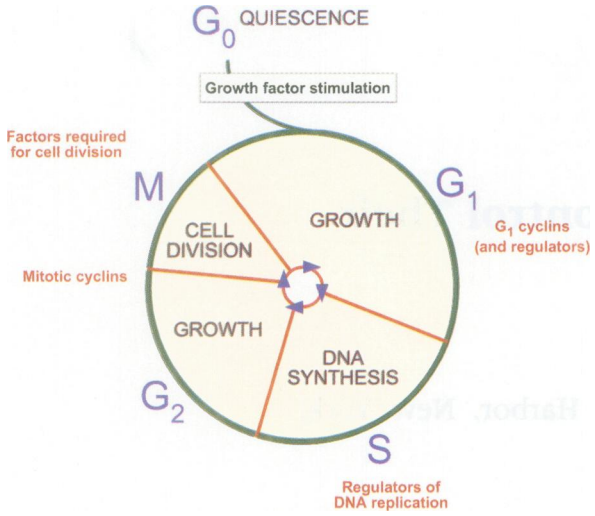

Fig. 1. The cell cycle. The diagram shows a schematic of the eukaryotic cell cycle. Shown are the five stages of the cell cycle, the key events that occur within each stage, and the key regulatory molecules involved. See text for details.

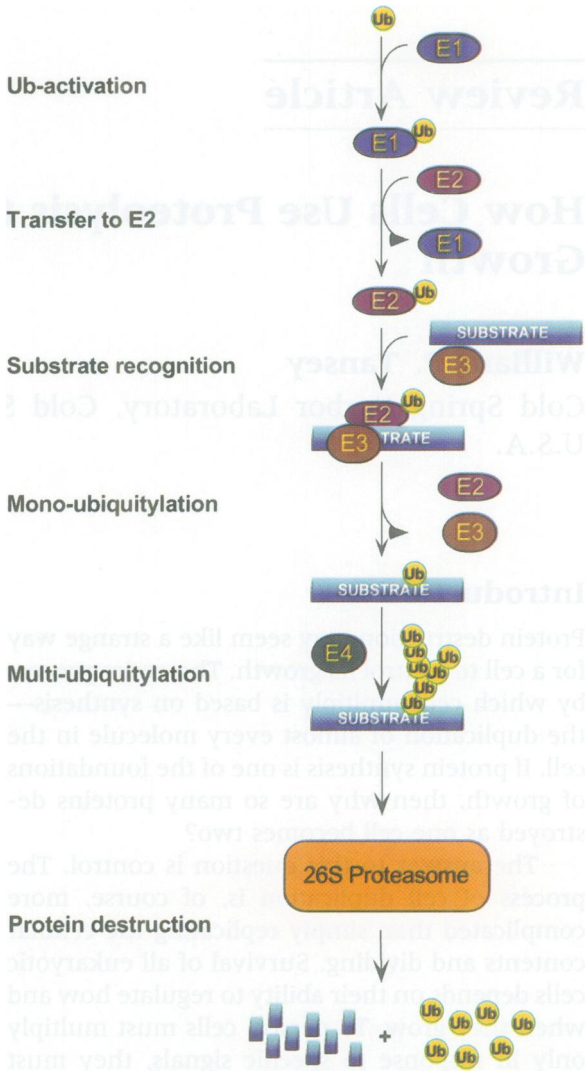

Fig. 2. Ubiquitin-mediated proteolysis. The diagram depicts the four classes of enzymes involved in transferring ubiquitin to target proteins. Note that the E1–E4 nomenclature is generic and that there are, for example, multiple E2 and E3 activities that are usually composed of many subunits. See text for details.

of proteolysis in growth control will come into focus.

Controlling Growth Through the Cell Cycle

How does one eukaryotic cell become two? In all eukaryotic cells, the process of duplication occurs within the context of a cell cycle—an ordered series of events through which each cell must pass in order to replicate. In the cell cycle, thousands of biochemical reactions are corralled into distinct groups that proceed in an ordered sequence. Each group of reactions in turn defines a distinct stage of cell cycle progression. Because passage from one stage of the cell cycle to the next occurs in response to specific signals, such as those generated by the presence of growth factors, the existence of the cell cycle allows cells to maintain tight control over their growth.

A simplified view of the cell cycle, with the five stages of cell cycle progression, is shown in Figure 1. In absence of growth factors, cells do not cycle and are said to be quiescent, or in the G_0 phase. In the presence of growth factors, cells enter the cell cycle at the stage known as G_1, and prepare for duplication of their DNA. Late in G_1, and in response to specific signals from the environment, cells commit to undergoing an entire cell cycle. After this commitment is made, cells then enter S phase, during which time DNA replication occurs, followed by another phase of growth and reorganization, known as G_2. If no errors in the replication process are detected, cells then enter the last stage of the cell cycle; mitosis, or M phase—the point at which duplicated chromosomes are neatly segregated into two daughter cells.

At its simplest level, the cell cycle is controlled by periodic fluctuations in the abundance of proteins known as cyclins. Each stage of cell cycle progression is defined by the expression of particular cyclins that control stage-specific events by activating particular kinases. For example, human cyclin D1 is expressed only in the G_1 phase of the cell cycle (see ref. 1 for review).

The presence of cyclin D1 activates cyclin-dependent kinases (CDKs) 4 and 6, which in turn phosphorylate the retinoblastoma protein Rb. Phosphorylation of Rb liberates active E2F, a transcription factor that activates (among other things) the expression of genes required for DNA synthesis (2). Because cyclin D1 expression is limited to a narrow window of time within G_1, these E2F-activated genes are expressed only when their products are needed, during the upcoming S phase, and not at other stages in the cell cycle. Similar regulatory cascades are initiated at other points by other cyclins.

Although cyclins characterize each stage of the cell cycle, they alone do not govern cell cycle progression, for cyclins themselves are regulated. Their synthesis is tightly controlled by the action of transcription factors, and their activity is tightly controlled by the action of regulatory proteins (e.g., CDK inhibitors, which mask the activity of cyclin–CDK complexes; reviewed in ref. 3). Moreover, cyclins work by signaling to appropriate subsets of effector molecules: transcription factors, regulators of DNA replication, and factors required for specific events in the cell cycle, like sister chromatid separation. In addition to cyclins, therefore, there are many molecules that have an impact on cell cycle progression, and there are thus many molecules whose activity must be precisely regulated for normal cell cycle progression to occur. As discussed below, proteolysis plays an important role in regulating the activity of these molecules.

Before ending this brief review of the cell cycle, it is worth noting that, although the details may vary from one species to the next, the same basic strategy for organizing and executing the events of cell duplication is used by all eukaryotes. Indeed, the fundamental conservation of this process in yeast to frogs to humans has been instrumental to our understanding of how and why cells cycle, and the role that proteolysis plays in this process.

The Ubiquitin–Proteasome Pathway of Protein Destruction

Cells can destroy proteins in many different ways. A typical budding yeast cell, for example, has well over 20 proteases at its disposal. Despite this battery of different proteolytic activities, however, one process—ubiquitin (Ub)-mediated proteolysis—dominates the control of cell growth. Almost all of the unstable proteins that regulate eukaryotic cell growth are destroyed by this unique pathway of protein breakdown.

Ub-mediated proteolysis is a process in which proteins are first tagged by covalent linkage to Ub,—a small, highly conserved protein—and then destroyed by the proteasome—a large, multicatalytic protease complex (for reviews see refs. 4 and 5). A general scheme for the Ub–proteasome pathway is shown in Figure 2. The process begins when Ub forms a thioester bond with a Ub-activating enzyme, or E1. Ub is then transferred to a Ub-conjugating enzyme, or E2. The E2, either alone or in conjunction with a Ub-protein ligase (E3), recognizes a specific element in the target protein called a degron (4). Once the degron has been recognized, Ub is transferred to a lysine residue somewhere on the target protein. Repeated rounds of this process [perhaps catalyzed by a Ub-chain assembly factor, E4 (6)] results in a highly ubiquitylated target protein that is destroyed by the proteasome.

There are three reasons why Ub-mediated proteolysis features prominently in the regulation of cell cycle progression. First, Ub-mediated proteolysis is highly selective. For a protein to be destroyed by this pathway, it generally must carry a degron to signal its ubiquitylation. This selectivity means that, from within a collection of regulatory molecules, specific proteins can be singled out for destruction as needed. Second, Ub-mediated proteolysis can destroy many proteins. Multiple E2 and E3 complexes participate in the pathway, each of which ubiquitylate a distinct set of target proteins. This diversity allows the Ub–proteasome pathway to destroy all of the different proteins needed for coordinating cell cycle progression. Finally, Ub-mediated proteolysis can be precisely controlled. Interactions between proteins and their respective E2/E3 complexes can be regulated, so that these proteins are destroyed only in response to specific signals. This control means that, even if a protein contains a degron, it is not always unstable, but rather can be destroyed at specific points in the cell cycle. Together, these three unique attributes—selectivity, diversity, and controllability—make Ub-mediated proteolysis ideally suited to regulating complex events like cell cycle control.

Targets of Ubiquitin-Mediated Proteolysis

As a final approach to understanding how proteolysis is used to control growth, we must identify the proteins that are targeted for destruction. Table 1 shows a list of proteins that are involved

Table 1. Cell cycle–related targets of ubiquitin-mediated proteolysis

Target	Function	Species	E2/E3
Cell cycle kinases and their regulators			
Cyclin D1	G_1 cyclin	*Hs*	SCF
Cyclin E	G_1 cyclin	*Hs*	SCF
Cln1	G_1 cyclin	*Sc*	SCF
Cln2	G_1 cyclin	*Sc*	SCF
Cln3	G_1 cyclin	*Sc*	SCF
Clb5	S-phase cyclin	*Sc*	APC
NIME	Mitotic cyclin	*An*	APC
Clb2	Mitotic cyclin	*Sc*	APC
Clb3	Mitotic cyclin	*Sc*	APC
Cdc13	Mitotic cyclin	*Sp*	APC
Cyclin A	Mitotic cyclin	*Ss*	APC
Cyclin B1	Mitotic cyclin	*Xl*	APC
Cyclin B3	Mitotic cyclin	*Xl*	APC
p21	CDK inhibitor	*Hs*	SCF
p27	CDK inhibitor	*Hs*	Ubc2/Ubc3
Far1	CDK inhibitor	*Sc*	SCF
Sic1	CDK inhibitor	*Sc*	SCF
Swe1	CDK inhibitor	*Sc*	SCF
Rum1	CDK inhibitor	*Sp*	SCF
NIMA	Mitotic kinase	*An*	APC
Plk1	Mitotic kinase	*Hs*	APC
Cdc5	Mitotic kinase/APC regulator	*Sc*	APC
Wee1	Mitotic inhibitory kinase	*Xl*	SCF
Cdc25	Mitotic phosphatase	*Sp*	Public
Regulators of DNA replication			
Cdc6	Replication initiation	*Sc*	SCF
Cdc18	Replication initiation	*Sp*	SCF
Geminin	Replication inhibitor	*Xl*	APC
Factors required for mechanics of cell duplication and division			
Pds1	Sister chromatid separation	*Sc*	APC
Cut2	Sister chromatid separation	*Sp*	APC
P58ctf13	Kinetochore assembly	*Sc*	SCF
Ase1	Mitotic spindle component	*Sp*	APC
Gic1	Cytoskeletal rearrangement	*Sc*	SCF
Gic2	Cytoskeletal rearrangement	*Sc*	SCF
Regulators of transcription			
DP1	Transcription factor/E2F partner		?
E2F-1	Transcription factor	*Hs*	SCF
Fos	Transcription factor	*Hs*	?
Jun	Transcription factor	*Hs*	?
Myc	Transcription factor	*Hs*	?
p53	Transcription factor	*Hs*	?
Mdm2	p53 antagonist	*Hs*	?
Components of proteolytic machinery and their regulators			
Cdc20	APC regulator	*Sc*	APC
Cdc4	SCF component	*Sc*	SCF
Grr1	SCF component	*Sc*	SCF

CDK, cyclin-dependent kinase; *Hs, Homo sapiens; Sc, Saccharomyces cerevisiae; An, Aspergillus nidulans; Sp, Schizosaccharomyces pombe; Ss, Spisula solidissima; Xl, Xenopus laevis;* SCF, Skp-Cullin–F-box complex; APC, anaphase-promoting complex.

in the control of cell growth and that are destroyed by Ub-mediated proteolysis. Table 1 also lists, where known, the E2/E3 complexes that ubiquitylate these proteins.

The first feature to emerge from Table 1 is the diversity of growth-related proteins that are destroyed by Ub-mediated proteolysis. There are 40-odd entries in Table 1, which together include all of the major categories of proteins needed to drive cell cycle progression. There are (*i*) cell cycle kinases and their regulators, which the signal events require for cell duplication; (*ii*) regulators of DNA replication, which maintain normal cell ploidy by limiting DNA replication to once per cell cycle; (*iii*) factors required for the mechanics of cell duplication, which drive the structural rearrangements needed to produce a daughter cell; (*iv*) regulators of transcription, which carry out growth-regulated patterns of gene synthesis; and (*v*) components of the proteolytic machinery itself, which regulate all aspects of cell cycle progression. Confronted with this list, it is difficult to imagine a type of growth-related protein that is not represented here. The number and breadth of these proteins illustrates the importance of Ub-mediated proteolysis to almost all aspects of eukaryotic growth control.

The second feature to emerge from Table 1 is that most of these proteins are ubiquitylated by one of two types of E2/E3 complexes; either an anaphase promoting complex (APC) (7) or a Skp-Cullin–F-box (SCF) complex (8). Detailed discussion of the architecture, function, and regulation of APC and SCF complexes is beyond the scope of this review (see refs. 9 and 10 for reviews on the APC and SCF complexes, respectively). But some features are worth considering here. First, the APC and SCF are large, multisubunit complexes. Some forms of the APC, for example, contain well over a dozen proteins, with a predicted molecular weight of 750,000 kD (see ref. 9 for a list of APC subunits). The intricate nature of these complexes implies that their activity is tightly regulated, because many gene products must be present in the right place and at the right time for an active APC or SCF complex to assemble. Second, APC and SCF exist in multiple forms (11). Subtle variations in APC or SCF subunit composition can create complexes that either ubiquitylate different target proteins or respond to different regulatory signals. There is thus no one APC or SCF, but a rather a collection of these activities, the members of which perform specialized tasks. Finally, although APC and SCF are evolutionarily related complexes (12) that have the same enzymatic activity (i.e., protein ubiquitylation), they tend to differ in the types of proteins they ubiquitylate. The APC is activated at the metaphase-to-anaphase transition, and thus plays a predominant role in destroying proteins that regulate exit from mitosis. SCF activity, in contrast, appears to be directed more toward proteins involved in the G_1-to-S transition—proteins such as the G_1 cyclins [e.g., Cln2p in *Saccharomyces cerevisiae* (8)] and their inhibitors [e.g., the human cyclin-dependent kinase inhibitor p27 (13)]. Thus these two types of ubiquitylation machines have evolved to carry out distinct functions in the regulation of cell growth.

A final point to be made from Table 1 is the broad range of species represented. Indeed, it is fair to say that probably all eukaryotes use Ub-mediated proteolysis to control their growth. Across the broad spectrum of eukaryotic life, therefore, the functional conservation of the cell cycle machinery is mirrored in the conserved way in which these proteins are regulated by destruction.

Putting Proteolysis to Work

How do cells use proteolysis to control their growth? One answer to this question has already been provided: by regulating the proteins that control cell cycle progression. But a more fundamental answer to this question needs to address exactly how the regulation of these proteins occurs. In other words, what strategies has the cell devised to harness the power of proteolysis for growth control? After all of the complexity that has preceded this point, the reader will be relieved to learn that the answer to this question is simple: there are just three ways in which Ub-mediated proteolysis is used to regulate cell growth. These basic strategies—timed destruction, continuous destruction, and regulated destruction—are at the heart of all uses of Ub-mediated proteolysis in the control of cell growth.

Using Proteolysis I: Timed Destruction

Because Ub-mediated proteolysis can be tightly regulated, it can bring about protein destruction at a very specific point during cell cycle progression. In response to a predetermined signal, Ub-mediated proteolysis can attack a specific set of target proteins, causing their levels to drop al-

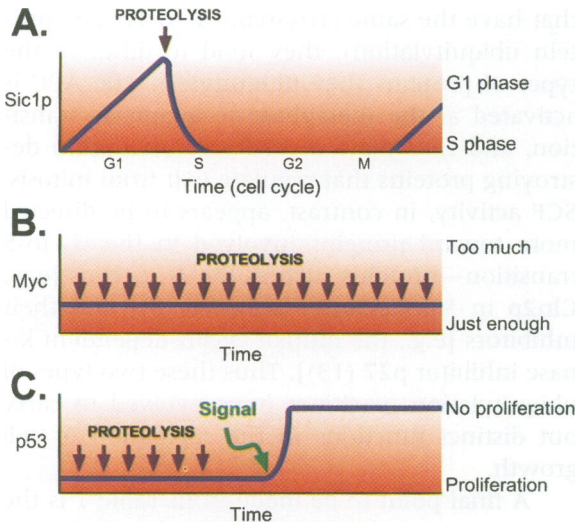

Fig. 3. How cells use proteolysis to control their growth. Three basic strategies for using Ub-mediated proteolysis are shown. (A) Proteolysis is used to destroy a protein at a very specific point in the cell cycle. In this case, timed destruction of the S-phase inhibitor Sic1p allows cells to pass through the G_1-to-S transition. (B) Proteolysis is used to keep the levels of a regulatory molecule low, and coupled to its synthesis. In this case, the continuous destruction of the oncoprotein Myc, coupled with relatively low rates of transcription of the *Myc* gene, keeps intracellular levels of Myc at a level compatible with controlled cell growth. (C) Regulated destruction is used to induce a rapid change in protein levels in response to a signal. In this case, DNA damage by UV light brings about a cessation of p53 destruction, resulting in a rapid rise in p53 levels and a halt to cell proliferation. See text for more details.

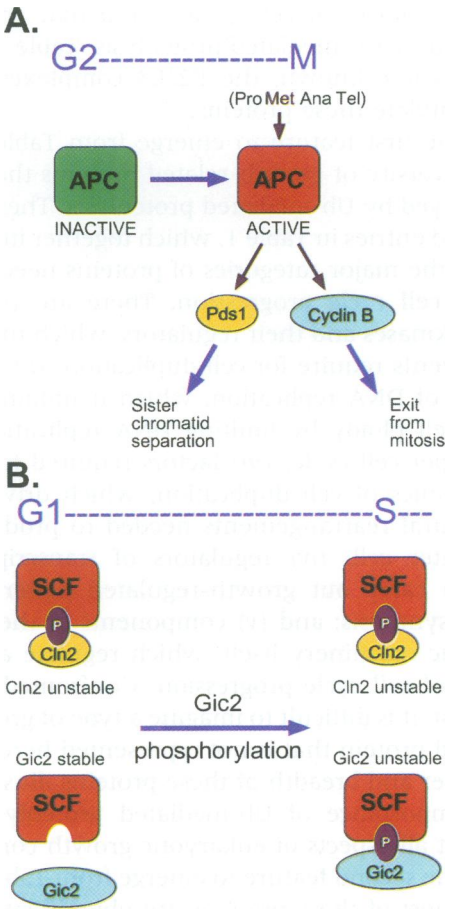

Fig. 4. Regulating proteolysis. The figure shows how Ub-mediated proteolysis can be regulated by either regulating the activity of the E3 (A) or regulating the susceptibility of the target protein (B). (A) Activation of the APC during metaphase signals destruction of multiple proteins such as Pds1p and the B cyclins; the coordinated destruction of these proteins is used to drive multiple molecular events required for exit from mitosis. (B) Differential phosphorylation of the G_1 cyclin Cln2p versus the Cdc42 effector Gic2p differentially affects their destruction by a common SCF complex: Cln2 is phosphorylated and unstable throughout G_1, while Gic2p is phosphorylated, and destroyed, approaching the G_1-to-S transition. See text for details.

most instantly. This strategy is used to define fixed points in the process of cell duplication.

One example of this use of proteolysis, presented in Figure 3A, is destruction of the yeast protein Sic1p (14). Sic1p is a CDK inhibitor that accumulates during G_1 and inhibits the action of cyclin–CDK complexes required for S-phase initiation. Toward the end of G_1, however, Sic1p is rapidly destroyed via SCF-mediated ubiquitylation. The resulting drop in Sic1p levels activates cyclin–CDK complexes required for S-phase initiation, and cells are propelled from one phase of the cell cycle to the next. This use of proteolysis occurs whenever regulatory proteins must disappear at a precise point: for example, destruction of the mitotic cyclins (15), which control exit from mitosis; destruction of Pds1p (16), which controls sister chromatid segregation; and destruction of geminin, which times the onset of DNA replication (17). The strategy of timed destruction provides perhaps the most dramatic demonstration of how proteolysis brings order and direction to the processes involved in cell duplication.

Using Proteolysis II: Continuous Destruction

A less spectacular, but nonetheless important, function of Ub-mediated proteolysis is the continuous destruction of regulatory proteins. Con-

tinuous proteolysis is a particularly important regulatory strategy because it serves to couple the steady-state levels of a protein to the rate of its synthesis. If a protein is always destroyed soon after it is made, it will disappear quickly once transcription of its gene is shut off. This strategy is illustrated in Figure 3B.

An interesting example of this use of proteolysis is observed with the oncoprotein Myc, which is a transcription factor that directly activates the expression of genes required for cell cycle progression [e.g., cdc25 phosphatase (18)]. Because of this direct link to the cell cycle, high levels of the Myc protein can drive a cell toward uncontrolled growth (19). Numerous mechanisms are thus in place to keep levels of the Myc protein tightly in check. For example, transcription of the *Myc* gene is highly controlled, as is the stability of Myc mRNA and translation of the Myc protein (see ref. 20 for details). Capping off all of this regulation is Ub-mediated proteolysis, which destroys the Myc protein within minutes of its production (21). In this scenario, the rapid destruction of Myc plays a pivotal role in keeping Myc under control, because it enables the preceding regulatory processes to exert their influence over Myc protein levels. This strategy of continuous destruction is used extensively in growth control—regulating transcription factors, G_1 cyclins (22), and components of the proteolytic machinery [e.g., the APC-activator Cdc20p (23)]—as a way to amplify and enforce the action of other regulatory mechanisms.

Using Proteolysis III: Regulated Destruction

An important feature of the eukaryotic cell cycle is that it can react to problems that arise during the process of cell duplication. If, for example, there has been a problem with the duplication of the DNA, cell cycle progression can be delayed until this problem has been corrected. This reaction of the cell cycle machinery protects the integrity of the genetic information, by ensuring that errors in the duplication process are not passed on to daughter cells. Proteolysis plays an important role in this response by its ability to execute rapid, signal-induced changes in the levels of regulatory molecules within the cell. This process of regulated destruction is the third way that cells use proteolysis to control their growth.

An interesting example of this kind of regulation, depicted in Figure 3C, involves changes in the destruction of the tumor suppressor protein p53 in response to DNA damage (24). p53 is normally an unstable protein that is turned over by Ub-mediated proteolysis. Upon exposure of cells to UV light, however, the destruction of p53 is quickly blocked (25), resulting in a rapid accumulation of p53, an induction of *p53* target genes, and a block to cell proliferation. Similar DNA damage and checkpoint-induced changes in proteolysis have been reported for the CDK inhibitor p21 (26), the mitotic kinase Wee1 (27), and the anaphase inhibitor Pds1p (28). By shutting proteolysis down when there is a problem, cells thus ensure that they divide only when they can produce normal, healthy daughters.

Regulating Ubiquitin-Mediated Proteolysis

A key aspect of protein destruction by Ub-mediated proteolysis is that it can be precisely regulated. Indeed, without such regulation, Ub-mediated proteolysis would not feature so extensively in the control of cell growth. There are many ways in which Ub-mediated proteolysis is regulated, but again, these can be reduced to just two simple principles: regulating the activity of the ubiquitylating enzyme (E3) or regulating the susceptibility of the target protein. These two modes of regulation are illustrated in Figure 4.

Regulating E3 Activity

If two proteins are ubiquitylated by the same E3, in the absence of any other forms of regulation, they will be destroyed in the same manner. When the E3 is inactive, the two proteins will be stable. When the E3 is active, the two proteins will be destroyed. This simple scenario illustrates how cells can coordinately destroy specific groups of proteins by regulating E3 activity.

A good example of this form of regulation is the APC, which becomes active late in mitosis. The activation of the APC promotes coordinate destruction of many proteins required for exit from mitosis. For example, Pds1p is ubiquitylated by a form of the APC containing the regulatory subunit Cdc20p (29). As shown in Figure 4A, Pds1p and APC^{Cdc20} apparently coexist throughout much of the cell cycle without incident. Approaching the metaphase-to-anaphase transition, however, APC^{Cdc20} is activated. The active APC^{Cdc20} then targets destruction of Pds1p, which in turn initiates a major event in mitosis—the segregation of sister chromatids. Importantly, Pds1p is not the only target of APC^{Cdc20}. As

shown in the figure, the activated APC^{Cdc20} also targets B-type cyclins (30) for destruction at this point, triggering other events required for exit from mitosis. Thus, by regulating APC^{Cdc20} activity, rather than individually regulating Pds1p and cyclin B susceptibility, the cell is able to efficiently coordinate multiple molecular events required for the anaphase-to-metaphase transition.

Regulating Substrate Susceptibility

If two proteins are ubiquitylated by the same E3, in the absence of any other forms of regulation, they will be destroyed in the same manner. If, however, the susceptibility of one of these proteins to ubiquitylation is differentially regulated, the two proteins will display very different stabilities. This simple scenario demonstrates how regulation of substrate susceptibility allows cells to selectively target a very specific and controlled subset of proteins for destruction.

The G_1 cyclin Cln2p and the Cdc42-effector Gic2p provide an interesting example of how substrate regulation allows for differential destruction of proteins via a common E3 (Fig. 4B). An SCF complex, containing the F-box protein Grr1p, targets the ubiquitylation of both Cln2p (8) and Gic2p (31). In both cases, substrate recognition by Grr1p requires phosphorylation of residues within the Cln2p and Gic2p degrons. Because these residues in the Cln2p and Gic2p degrons are differentially phosphorylated, the two target proteins are destroyed in different ways: Cln2p is continuously destroyed during the G_1 phase (22), whereas Gic2p accumulates during G_1, but is rapidly destroyed following the G_1–S transition (31). In the case of Cln2p, continuous destruction serves to link levels of the Cln2p protein to the rates of Cln2 transcription; in the case of Gic2p, timed destruction of Gic2p restricts cytoskeletal polarization to the G_1 phase of the cell cycle. Thus, by regulating individual substrate susceptibilities, the cell is able to use the same SCF^{Grr1} complex in different ways and at different times to drive diverse events required for cell duplication.

Ubiquitin-Mediated Proteolysis and Cancer

As discussed throughout this review, Ub-mediated proteolysis regulates many proteins involved in growth control. It seems reasonable to conclude, therefore, that dysregulation of Ub-mediated proteolysis might lead to uncontrolled cell proliferation and perhaps cancer. This is indeed the case, and although research in this area is still in its infancy, we are beginning to understand how changes in protein destruction feature in oncogenesis.

First, changes in Ub-mediated proteolysis are important for the action of several viral oncoproteins. The oncogenic form of Jun, for example, carries a deletion that removes much of the Jun degron (32), making v-Jun more stable than its cellular counterpart. A similar stabilizing deletion has been described for the transcription factor v-Myb (33). In another example, the human papillomavirus (HPV16) oncoprotein E6 specifically targets the destruction of the tumor suppressor protein p53 (34). The E6-mediated destruction of p53, which defeats the cell's ability to arrest or die in response to oncogenic pressure, is an essential part of HPV16's strategy for directing cellular transformation. Given the uncanny ability of tumor viruses to select for the most important and vulnerable regulatory processes, it seems likely that other, similar examples will be uncovered in the future.

Second, changes in the Ub-mediated destruction of cell cycle regulators have been directly associated with cancer. The best examples of this to date are p27 and Myc, which illustrate how either up-regulation or down-regulation through Ub-mediated proteolysis can cause problems. Ub-mediated proteolysis of the CDK inhibitor p27 is increased in some human cancers (35), resulting in a substantial decrease in steady-state p27 levels. This Ub-mediated decrease in p27 levels generally predicts poor patient survival. Conversely, Ub-mediated destruction of Myc is down-regulated in some malignancies; the Myc degron is a hotspot for mutations in a variety of leukemias and lymphomas (36), and several of these mutations have been shown to subvert the Ub-mediated destruction of Myc (21), resulting in the production of a significantly more stable protein with increased transforming ability. Other examples of substrate-specific defects in proteasomal destruction will undoubtedly be uncovered in the future.

Finally, there is one example of how mutations in a component of the Ub–proteasome pathway can feature directly in cancer. The human tre-2 oncogene encodes a deubiquitylating enzyme (37) that removes Ub groups from substrate proteins during their destruction by the proteasome. Because the yeast homolog of this

gene, DOA4, is required for the efficient destruction of multiple, but not all, ubiquitylated substrates, it is reasonable to speculate that tre-2 is oncogenic because it mediates the destruction of a select group of growth-related proteins in mammalian cells. These proteins are yet to be identified. Although this last example is interesting, it seems unlikely that situations like this, where the proteolytic machinery itself is deregulated, will feature extensively in cancer. The involvement of Ub-mediated proteolysis in many aspects of cellular metabolism may 'protect' the ubiquitylation machinery from deregulation, because changes in this machinery might simply kill cells, rather than offer them a growth advantage. On balance, therefore, it seems much more probable that substrate-specific changes in protein destruction, as outlined above, will dominate the arena of Ub-mediated proteolysis and cancer.

Perspectives

It is clear that Ub-mediated protein destruction permeates all aspects of cell cycle control in all eukaryotes. The multifaceted use of this pathway and its evolutionary success are testaments to the unique suitability of Ub-mediated proteolysis to the control of cell growth. In recent years, biochemistry and genetics have come together to fuel an explosion in our understanding of how and why proteolysis controls cell growth. But many questions remain. What is the full extent of the involvement of the Ub–proteasome pathway in the control of the cell cycle? Although many substrates have been identified, it seems likely that we have only scratched the surface. We need to identify the complete collection of target proteins and all the ubiquitylating complexes, and we need to understand how interactions between them are controlled. We also need to understand the extent of cross-talk between different ubiquitylation complexes. The potential for cross-talk is high, given the fact that E3's often contain common subunits and usually target multiple proteins for destruction. For example, can a protein be stabilized if its E3 is actively involved in ubiquitylating another protein? Is this cross-talk used to control cell growth? Finally, we must understand not only how the Ub–proteasome pathway functions in cell cycle control but also how other proteases feature in this process. There is a small but growing group of proteases that target cell cycle regulatory proteins—Tsap, for example, which cleaves cyclin A (38)—and a recently identified complex that can substitute for the essential functions of the proteasome (for review see ref. 39). We must understand the role of these proteases and how they work with Ub-mediated proteolysis to control cell growth. There is clearly much to be done: it's degrading work, but someone's got to do it.

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